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Dynamic computer simulation of *Clostridium perfringens* growth in cooked ground beef☆

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Abstract

The objective of this study was to develop a computer simulation algorithm to dynamically estimate and predict the growth of *Clostridium perfringens* in cooked ground beef. The computational algorithm was based on the implicit form of the Gompertz model, the growth kinetics of *C. perfringens* in cooked ground beef, and the fourth-order Runge–Kutta numerical method. This algorithm was validated using a cocktail of three strains of *C. perfringens* spores grown under isothermal, square-waved, linear cooling, and exponential cooling temperature profiles. In general, the results of computer simulation matched closely with the experimental data with the absolute errors less than 0.5 $\log_{10}$ CFU/g. This method may be a useful tool for the food industry, regulatory agencies, distributors, and retailers to predict the effect of temperature abuse on the microbial safety of *C. perfringens* and other foodborne pathogens in processed meat products.
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Keywords: *Clostridium perfringens*; Numerical analysis; Dynamic simulation

1. Introduction

Clostridium perfringens is a Gram-positive spore-forming bacillus widely distributed in the environment (Granum, 1990), and it is one of the most common causes of foodborne illnesses in the United States (Shandera et al., 1983; Bean and Griffin, 1990). Cooked meat products, such as ham, roast beef, and corned beef, are frequently associated with foodborne outbreaks of *C. perfringens* gastroenteritis (Barnes et al., 1963; Hall and Angelotti, 1965; Gross et al., 1989; CDC, 1994). Consumption of food products contaminated with large numbers of vegetative cells of this organism can cause symptoms such as acute abdominal pain and diarrhea within 8–15 h after ingestion (FDA-CFSAN, 2001).

The spore form of *C. perfringens* is more resistant to heat than vegetative cells and can withstand heating at 100 °C for up to 1 h (Collee et al., 1961). As a result, the spores of *C. perfringens* can survive normal processing conditions for making processed meat products, such as ham, roast beef, and corned beef. Instead of being inactivated during cooking, as are vegetative cells, almost all the spores are activated by heat (Barnes et al., 1963). Since *C. perfringens* can grow in a wide temperature range between 10 and 52

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°C (FDA-CFSAN, 1998) and at high growth rates at temperatures between 30 and 47 °C (Craven, 1980; Juneja et al., 1994), the heat-activated spores of *C. perfringens* in finished meat products can become a public heath concern. If the temperatures of cooked meat products are not properly maintained, the spores of *C. perfringens* can germinate, outgrow, and actively multiply to dangerously high dose levels, posing a potential public health risk.

Food poisoning caused by *C. perfringens* has been historically linked to gross temperature abuse in finished foods (Gross et al., 1989; Foster, 1997; CDC, 1994). Rapid cooling and proper temperature control are critical to the prevention of foodborne *C. perfringens* gastroenteritis. The U.S. Department of Agriculture (USDA) requires that the internal temperature of the slowest cooling point in cooked beef, roast beef, and cooked corned beef should be cooled from 48.9 to 12.8 °C in 6 h or less, and the cooling should continue to 4.4 °C prior to boxing (USDA, 1993).

Process deviation and temperature abuse are practically inevitable in various real-world operations of cooling, distribution, storage, retail, and serving of cooked meat products. Assessing potential bacterial growth in cooked meat products undergoing dynamic (discrete or continuous) temperature changes has been a challenge for food microbiologists and food safety risk assessors. Most mathematical models available in predictive microbiology are kinetic models for estimating bacterial growth under constant temperature conditions (McDonald and Sun, 1999).

The objective of this project was to develop a computer simulation algorithm to dynamically estimate and predict growth of *C. perfringens* in cooked ground beef under both discrete (square-waved, for example) and continuous temperature changes. Such a computer algorithm will provide a real-time estimation and prediction of growth of this organism in cooked meat products, and prevent contaminated products from entering the market and being consumed.

2. Materials and methods

2.1. Test organisms and preparation of samples

Three strains of *C. perfringens* spores (NCTC 8238, NCTC 8239, and NCTC 10288) were chosen as test organisms in this study. These strains were provided by Dr. John Novak of USDA-ARS-ERRC located in Wyndmoor, PA. The spore crops were grown and harvested using the procedures developed by Juneja et al. (1993). Each spore crop was washed twice, resuspended in sterile deionized water, and maintained at 4 °C until use.

Ground beef (93% lean), purchased from a local grocery store, was irradiated to microbiological sterility using a ^{137}Cs γ-irradiation source available at USDA-ARS-ERRC. The irradiation of frozen ground beef was conducted at −30 °C to a final dose of 42 kGy (Thayer et al., 1995) to eradicate all contaminating microorganisms.

Aliquots of three *C. perfringens* spore strains were aseptically mixed into approximately 1500 g of irradiated ground beef in a Kitchen Aide Mixer (Model Max Watts 325). Mixing was conducted twice (30 min each) at medium speed and a homogeneous distribution of bacterial cells was experimentally verified. The initial inoculum was approximately 100 spores/g ground beef. The inoculated ground beef was divided into 5 ± 0.02 g portions and packaged into plastic filter bags (12×19 cm, Model BagPage® BP 100, Topac). Each plastic bag was vacuumed and sealed at a final vacuum level of 15 mm Hg. Beef samples were kept frozen until use.

2.2. Isothermal growth study

Isothermal growth studies were conducted to obtain the growth kinetics of *C. perfringens* in cooked ground beef. Frozen samples were thawed overnight in a refrigerator ($\approx$ 4 °C) and consequently heat-shocked at 75 °C in a water bath (Model ESRB-7, Techne, Princeton, NJ) for 20 min to activate *C. perfringens* spores and to kill any contaminating vegetative cells. After briefly rinsing the plastic bags containing heat-shocked beef samples with running water at room temperature ($\approx$ 20 °C), samples were placed into incubators maintained isothermally at 17, 25, 30, 36, 45, 47, and 50 °C, respectively. Samples from each incubation temperature were periodically removed from the incubators for determination of bacterial cell concentrations. Experiments were replicated at least three times for each isothermal growth curve.

2.3. Determination of C. perfringens cell concentration

Samples retrieved from incubators were immediately diluted with equal volumes (5 ml) of 0.1% sterile peptone water. Because heat shock at 75 °C caused beef protein to denature, a rubber hammer was used to gently break samples prior to subjecting the samples to a MiniMix Stomacher (Model BaxMix® 100W, InterScience). The ground beef samples were homogenized in the stomacher at maximum speed for 12 min. After homogenization, a small volume (0.1 ~ 0.5 ml) of the liquid portion was serially diluted with 0.1% sterile peptone water and plated on Shahidi-Ferguson Perfringens (SFP) agar. Each SFP agar plate was overlaid with approximately 10 ml of freshly prepared SFP agar. Upon the solidification of the SFP agar overlay, the plates were placed in an anaerobic chamber (Model Bactron IV, Sheldon Manufacturing, Cornelius, OR) and incubated for 24–48 h at 37 °C under an atmosphere of $CO_2/N_2/H_2$ (85%:10%:5%). Typical *C. perfringens* colonies were counted after incubation.

2.4. Dynamic growth study

To study the dynamic growth of *C. perfringens* in ground beef and validate the computer simulation algorithm, beef samples were incubated under three different temperature scenarios: (1) square-waved temperature profiles, (2) linear cooling, and (3) exponential cooling. In the tests of the first temperature scenario, two square-waved temperature profiles, alternating between 30 and 45 °C or 45 and 36 °C, were used to validate the computer algorithm. For each temperature profile, samples were alternated between two incubators maintained at 30 and 45 °C or 45 and 36 °C. At each transfer between two incubators, samples were taken to determine the cell concentrations using the procedures described in the previous section.

In both linear and exponential cooling experiments, samples were placed in a water bath (Model ESRB-7, Techne). The temperature of the water bath was controlled by a computer program developed for this study. In the linear cooling tests, the temperature of the water bath was initially set at 51 °C and changed at the rate of −0.1 °C/min. In the exponential cooling tests, the temperature of the water bath was controlled to change exponentially from 51 to 10 °C in 18 h. During exponential cooling, Eq. (1) was used to determine the water bath temperature. In this equation, T is the temperature of the water bath (°C), T_0 the initial temperature (°C), K the exponential cooling rate (0.09051/h), and t the incubation time (h). With the control algorithm developed in this study, very accurate temperature profiles were obtained in the experiments.

$$T = T_0 \exp(-Kt) \quad (1)$$

2.5. Automatic control of cooling temperatures

The water bath used in this study was manufactured by Techne. A temperature control unit (TU-20D) was supplied to control both heating and cooling of the unit (Fig. 1). A circulator was used to circulate the water and to achieve a uniform temperature distribution in the water bath. The cooling could be achieved by a water cooling coil and/or a built-in refrigeration system. An RTD sensor was used to monitor the temperature of the water bath. The heating of the water bath was controlled by a proportional-integral-derivative (PID) controller in the TU-20D unit. A PID control algorithm was built into the internal memory

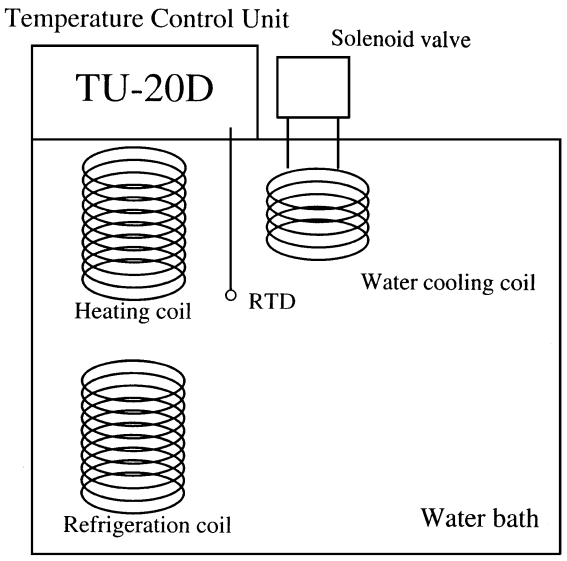

Fig. 1. A sketch diagram of refrigerated water bath.

of the controller and could not be manipulated. The manufacturer also supplied a PC-based temperature control program, ThermSoft, to allow programmable heating and cooling temperature controls.

However, the accuracy of the temperature control achieved by ThermSoft was not adequate for this study as substantial temperature deviations were observed during the initial testing of the unit.

A new PC-based temperature control program written in Microsoft Visual Basic (V6.0) was developed to dynamically control the temperature of the water bath. A control algorithm shown in Fig. 2 was used to control the cooling process. This new control program utilized an RS-232 communication port in the temperature control unit TU-20D to communicate between the serial port of a personal computer and the controller. At the interval of every 5 s, the control program sent a new set point signal to the control unit and received a signal of the real-time temperature of the water bath. Depending on the magnitude of the difference between the water bath temperature and the set point, commands were sent to the TU-20D unit to control the cooling water or the refrigerator. Preliminary testing of the control algorithm indicated that the temperature profiles were much more accurate that the one provided by Techne. The error of temperature control was less than 0.25 °C.

2.6. Isothermal growth models and effect of temperature on growth

The experimental growth data collected in the isothermal studies were used to develop mathematical growth models. To describe the sigmoid growth of bacteria under isothermal conditions, a modified Gompertz model (Gibson et al., 1987) was selected in this study. The plate counts of *C. perfringens* were converted to $\log_{10}$ values and analyzed by nonlinear regression to fit the experimental data to the Gompertz model (Eq. (2)). For each experiment, an independent growth model was developed. In Eq. (2), $L(t)$ is the $\log_{10}$ CFU/g of cell concentration, A and B are the initial and final cell concentrations, t the growth time, μ the relative growth rate at M, and M the time when the absolute growth rate is the maximum.

$$L(t) = A + (B - A)\exp\{-\exp[-\mu(t - M)]\} \quad (2)$$

A Windows-based statistical package, NCSS 2000 (Hintze, 1999), was used to analyze and fit the growth curves. During nonlinear regression, the Levenberg–Marquardt nonlinear least-squares algorithm (Nash, 1987) was used to obtain the parameters of the Gompertz model. A pseudo-R^2 was generated for each growth curve to approximate the usual R^2 used in linear regression. Although not an indicator of the

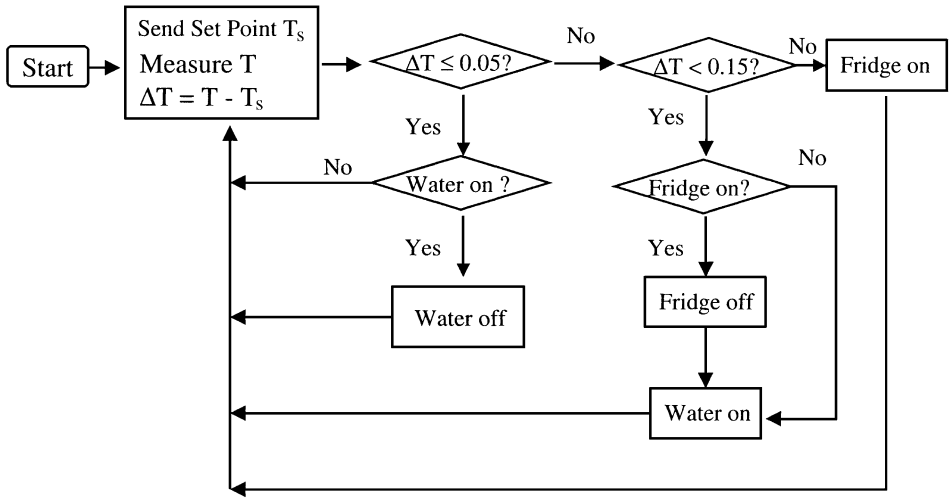

Fig. 2. A new temperature control algorithm for the refrigerated water bath.

goodness of fit, the pseudo-R^2 served well for comparative purpose.

A modified Ratkowsky equation (Eq. (3)) was used to analyze the effect of temperature on relative growth rate (μ) of the Gompertz model (Zwietering et al., 1991). In Eq. (3), a and b are constants. T_{min} and T_{max} are the theoretical minimum and maximum growth temperatures for *C. perfringens* in ground beef. These parameters were also obtained by nonlinear regression using NCSS 2000.

$$\mu = a(T - T_{min})^2\{1 - \exp[b(T - T_{max})]\} \quad (3)$$

2.7. Dynamic growth of C. perfringens

The Gompertz model (Eq. (2)) developed in the isothermal studies can successfully describe bacterial growth under constant temperature conditions (Gibson et al., 1987; McClure et al., 1994). In combination with the modified Ratkowsky equation (Eq. (3)), the effect of temperature on bacterial growth under isothermal conditions can be evaluated. However, these equations cannot be directly used to estimate bacterial growth under dynamic temperature conditions.

To evaluate bacterial growth under dynamic temperature conditions, the isothermal bacterial growth equation (Eq. (2)) can be differentiated (Van Impe et al., 1992, 1995) to produce an implicit form of the growth function. Differentiating against t in both sides of Eq. (2), bacterial growth can be expressed in a differential form (Eq. (4)), which can be further simplified and became a function of L (Eq. (5)).

$$\frac{dL}{dt} = \mu(B-A)\exp\{-\exp[-\mu(t-M)]\} \times \exp[-\mu(t-M)] \quad (4)$$

$$\frac{dL}{dt} = \mu(L-A)\ln\left(\frac{B-A}{L-A}\right) \quad (5)$$

The derivation of Eq. (5) was based on the isothermal Gompertz model. However, it can be applied to non-isothermal conditions with the application of the temperature-dependant variable μ. Under dynamic conditions with both μ and L varying with time, Eq. (5) cannot be solved analytically. Therefore a numerical technique must be used. In this study, the fourth-order Runge–Kutta method (Boyce and DiPrima, 1977) was applied to numerically solve the equation. During numerical analysis of bacterial growth under dynamic temperature conditions, the variable μ in Eq. (5) was supplied by the modified Ratkowsky equation (Eq. (3)).

An initial value of L must be supplied to Eq. (5) when the fourth-order Runge–Kutta method is used to solve for L. At $t=0$, L is equal to the initial cell concentration A. However, the equation becomes singular if $L(0)=A$ is used. To overcome this problem, a pseudo-initial value of L can be used to replace the real initial value (Van Impe et al., 1992, 1995). In this study, the pseudo-initial value was chosen using Eq. (6) with a very small ΔL_0 at $t=0$ (Eq. (6)).

$$L_0 = A + \Delta L_0 \quad (6)$$

A variable step-size, fourth-order Runge–Kutta numerical algorithm was developed using Microsoft Visual Basic (V 6.0). The time–temperature history of the samples and the bacterial growth kinetics were incorporated into this computer program for numerical simulation of the growth of *C. perfringens* in cooked ground beef under both static and dynamic temperature conditions.

3. Results and discussion

3.1. Isothermal growth kinetics

The spores of *C. perfringens* were homogeneously dispersed in the ground beef samples. The initial concentration of *C. perfringens* was 2.03 ± 0.05 $\log_{10}$ CFU/g (mean $\pm$ standard error). Growth of *C. perfringens* in cooked beef was sigmoidal and could be described by the isothermal Gompertz model (Fig. 3). Determined from the B values of all of the Gompertz curves, the average maximum concentration of *C. perfringens* in ground beef was 8.24 ± 0.07 $\log_{10}$ CFU/g. This value was adopted as the maximum cell concentration in this study. The relative growth rate, μ, was fitted to the modified Ratkowsky equation with a relatively high R^2 of 0.912 (Eq. (7)). Graphically this equation describes the general trend of μ as a function of temperature very well (Fig. 4). Based on the modified Ratkowsky equation, the minimum and maximum growth temperatures were approximately 10 and 51 °C. These two values were in close agree-

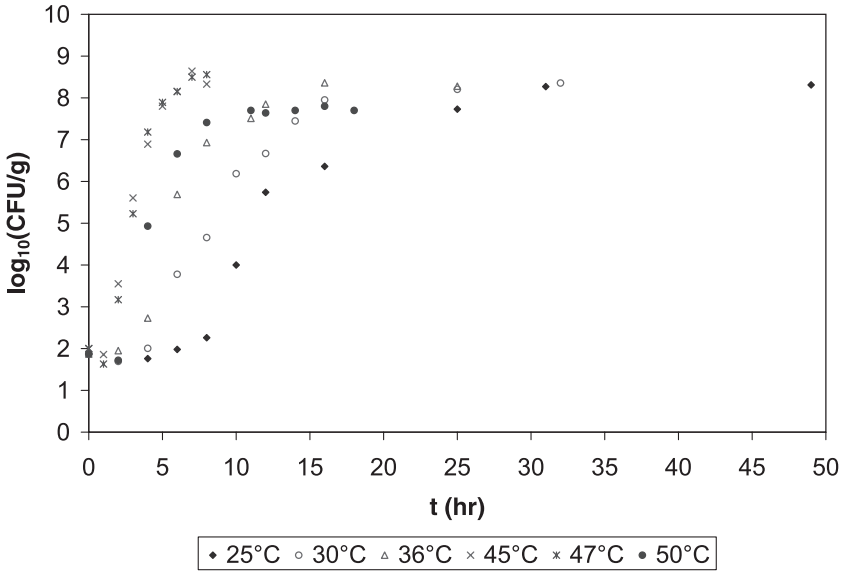

Fig. 3. Representative growth curves of *C. perfringens* in ground beef.

ment with the values published in the literature (FDA-CFSAN, 1998; Juneja et al., 1999).

$$\mu = 7.57 \times 10^{-4}(T - 10.52)^2 \times \{1 - \exp[1.007(T - 50.82)]\} \quad (7)$$

The implicit Gompertz equation (Eq. (5)) is a primary model and therefore can be used to describe the isothermal bacterial growth. It can be combined with the secondary model to describe the isothermal bacterial growth at any temperature. Fig. 5 depicts growth curves at three temperatures obtained from the numerical analysis of the implicit Gompertz equation. In all these curves, the initial value of ΔL_0 was 1.72×10^{-4} and the μ values were estimated from Eq. (7). Generally, the results of numerical analysis

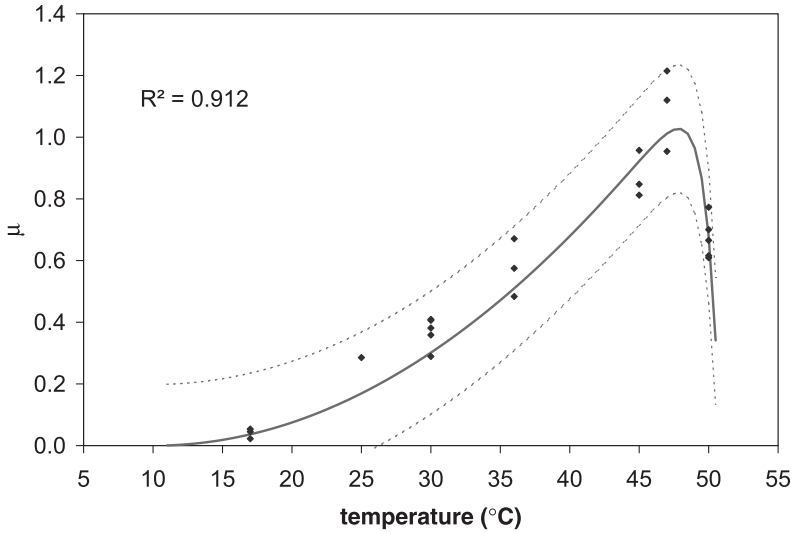

Fig. 4. Dependence of the relative growth rate (μ) on temperature as described by the modified Ratkowsky equation.

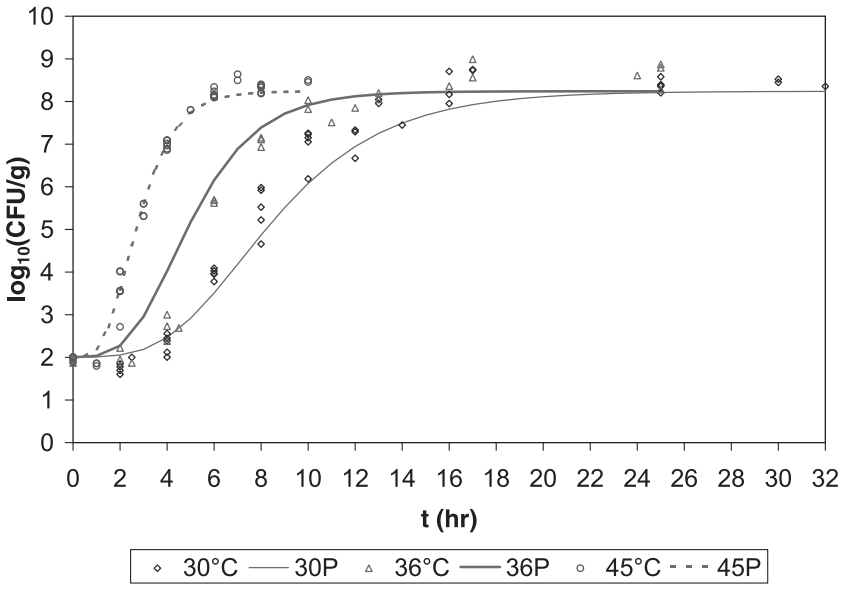

Fig. 5. Numerical estimation of the growth of *C. perfringens* in ground beef under isothermal conditions.

using the fourth-order Runge–Kutta method matched the experimental growth data very well.

In the implicit form of the Gompertz model, the term M is not included. Therefore, the lag phase time of a growth curve is not reflected in the implicit equation. However, selection of the initial ΔL_0 values affects the development of the lag phase in the simulated results. Fig. 6 shows the effect of ΔL_0 values on the shape of growth curves at 47 °C. Evidently, the lag phase of growth curves generated from the implicit Gompertz model decreases with increasing ΔL_0 values (Fig. 6). This interdependency is possibly caused by the numerical methodology since the Runge–Kutta method relies on the previous value to estimate the next result during iterations. The interdependency is also caused by the nature of the implicit form of the Gompertz equation where the initial value of L cannot be used directly to initiate the numerical iterations. In this study, an initial ΔL_0 value of 1.72×10^{-4} seemed adequate for most of the isothermal growth curves.

3.2. Dynamic simulation of bacterial growth under fluctuating temperature conditions

Temperature abuse is unavoidable throughout the chain of food production, distribution, storage, retail, or even consumption at home. Outbreaks of food-borne *C. perfringens* poisoning are primary a result of gross temperature abuse. Temperature fluctuation, represented by square-waved temperature profiles in this study, is a typical example of temperature abuse. The implicit Gompertz equation can be used to predict the extent of the growth of *C. perfringens* in the cooked meat products.

Figs. 7 and 8 demonstrate the computer simulation results of the dynamic growth of *C. perfringens* incubated under two different square-waved temperature profiles. The initial ΔL_0 value was 1.72×10^{-4} for both tests. Fig. 7 represents the dynamic growth of *C. perfringens* in cooked ground beef at temperatures fluctuating between 30 and 45 °C, staring at the lower temperature (30 °C). In Fig. 8, the temperature fluctuated between 45 and 36 °C, starting at the higher temperature (45 °C). The simulation results agreed with the experimental data very well in both experiments.

3.3. Dynamic simulation of bacterial growth under continuous temperature changes

Numerical analysis of the implicit Gompertz equation is particularly suitable for estimating the bacterial growth under continuous temperature changes (such as cooling). Under continuously changing temperature

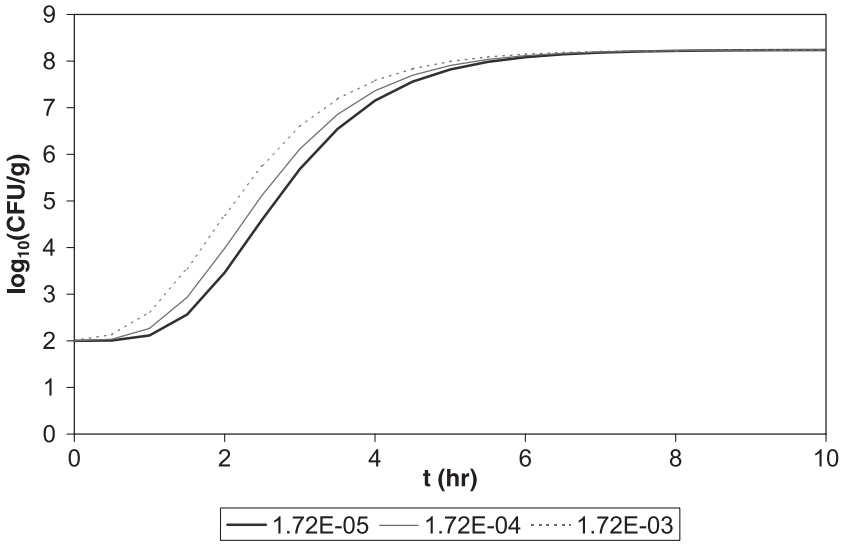

Fig. 6. Effect of the initial ΔL_0 value on the results of computer simulation.

conditions, the bacterial growth rate varies with both time and temperature. Therefore, bacterial growth cannot be estimated by traditional methods using the Gompertz model. During numerical analysis of the implicit Gompertz equation, small time steps must be used to estimate the bacterial growth.

Figs. 9 and 10 illustrate two example growth curves subjected to continuous temperature changes. Fig. 9 demonstrates the results of computer simulation of a process with a linear cooling profile. The experimental temperature was initially set at 51 °C, and was adjusted to decrease linearly to 10 °C at the rate of −0.1 °C/min. Fig. 10 depicts the simulation results of an exponential cooling profile with the growth temperature controlled to change exponentially from 51 to 10 °C in 18 h. During the computer

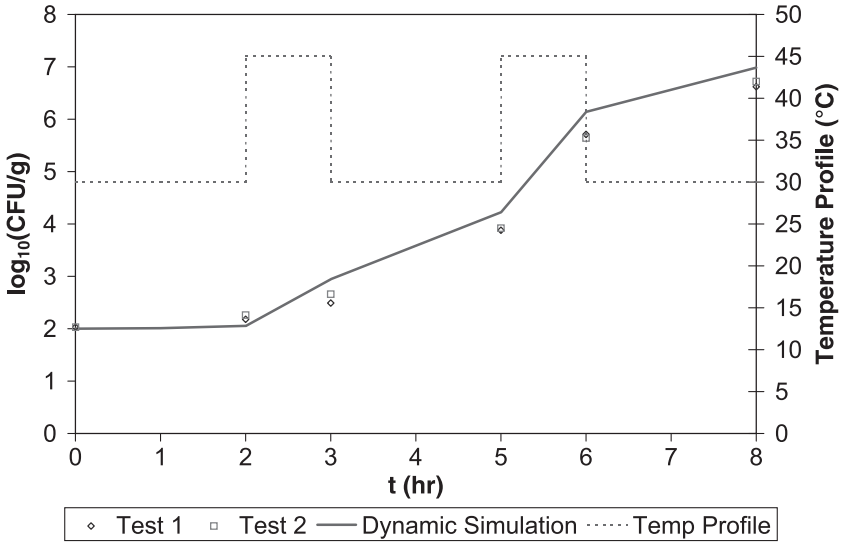

Fig. 7. Computer simulation of the growth of *C. perfringens* in ground beef with the incubation temperatures alternated between 30 and 45 °C.

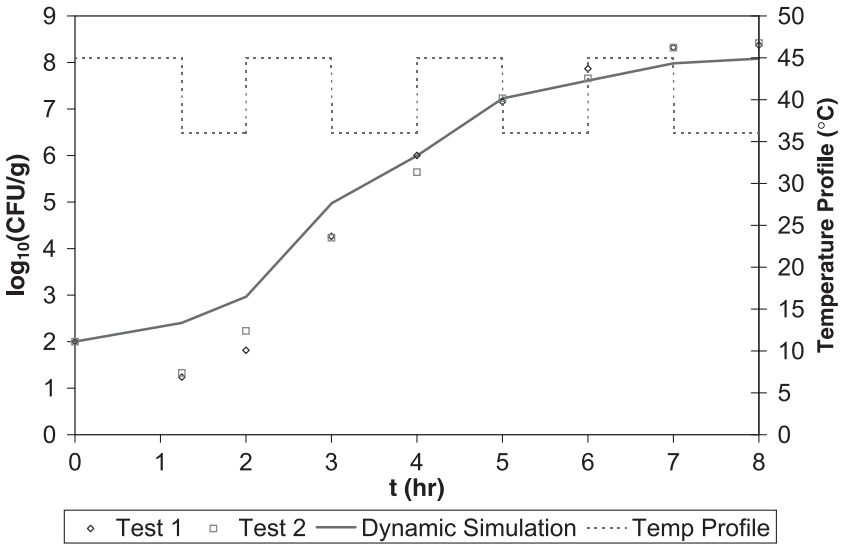

Fig. 8. Computer simulation of the growth of *C. perfringens* in ground beef with the incubation temperatures alternated between 45 and 36 °C.

simulation of the continuous temperature profiles, a much smaller initial ΔL_0 (2.15×10^{-9}) was used in the numerical iterations. The results of computer simulation matched the experimental data closely in both experiments.

Conventionally, the bacterial food spoilage process can be categorized into three different phases, including the lag, exponential, and stationary phases. Estimating the lag phase duration, exponential growth rate, and maximum bacterial growth of an isothermal process is fairly straightforward, and can be readily obtained from the explicit Gompertz model (Zwietering et al., 1990). However, the estimation of these parameters, particularly the lag phase duration, is difficult when the temperature changes continuously with time. The numerical analysis method can suc-

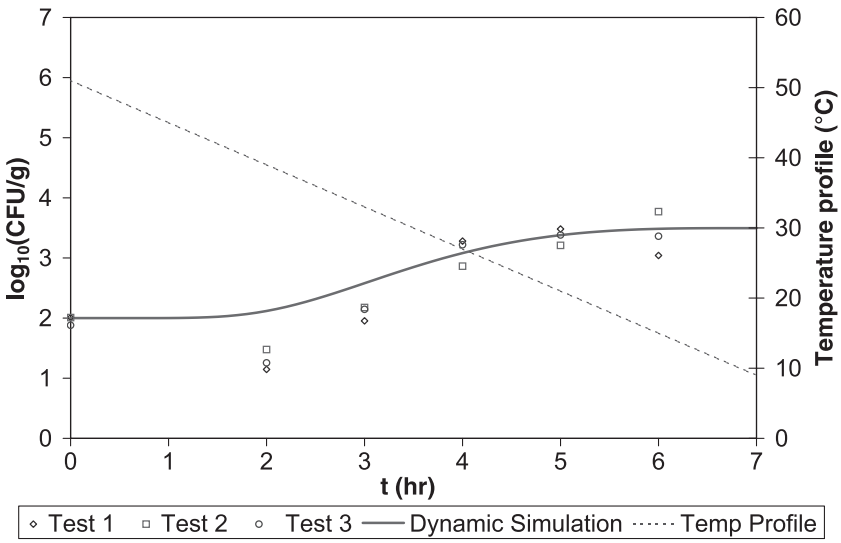

Fig. 9. Computer simulation of the growth of *C. perfringens* in ground beef with the incubation cooling linearly from 51 to 10 °C at 0.1 °C/min.

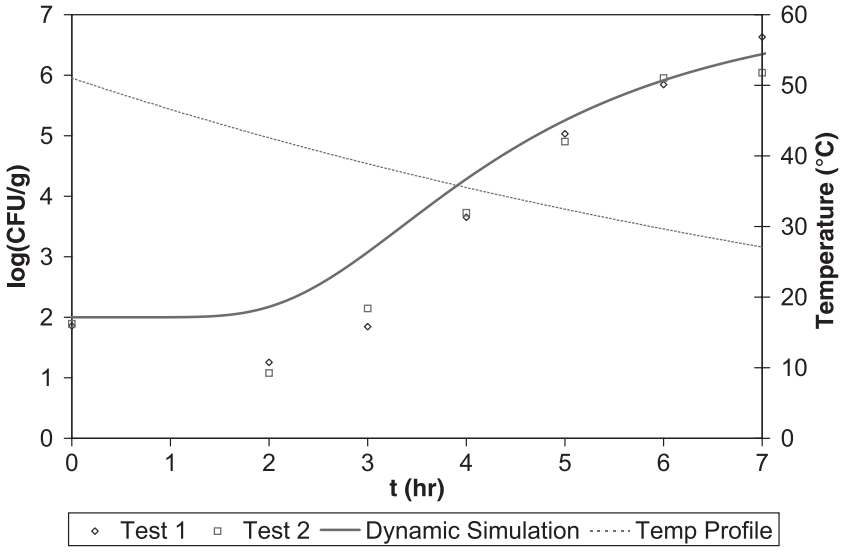

Fig. 10. Computer simulation of the growth of *C. perfringens* in ground beef with the incubation cooling exponentially linearly from 51 to 10 °C in 18 h.

cessfully provide a solution to estimate the entire bacterial growth process subject to a continuous change in the growth temperature. In both temperature conditions shown in Figs. 9 and 10, the lag phase times estimated by the numerical method were approximately 2 h.

In general, the absolute errors of the dynamic simulation of bacterial growth for various temperature profiles were less than 0.5 $\log_{10}$ CFU/g, except for a few data points located in the initial stages of incubation. The larger errors were primarily distributed within a small region, or 1–2 h after the completion of the lag phases. This region is typically located at the end of the lag phase and the early stage of the exponential phase. These errors, however, were not caused by computer simulation, but by the unique physiological behaviors of *C. perfringens*. The vegetative cells of *C. perfringens* may experience a sudden death, or Phoenix phenomenon, immediately after the incubation, particularly after being exposed to high temperature conditions (Shoemaker and Pierson, 1976). Such a phenomenon may complicate the computer simulation. However, these errors may be neglected since the bacterial counts are very small, and the computer simulation may provide a more conservative estimation in this region. For the most part of a growth curve, the results of computer simulation are accurate enough for general food safety applications.

4. Conclusion

The computer simulation algorithm based on the implicit form of the Gompertz equation, the growth kinetics of *C. perfringens*, and the fourth-order Runge–Kutta numerical analysis method was developed and tested in this study. This computer algorithm was applied to estimate the cell populations of *C. perfringens* grown in cooked ground beef undergoing various temperature environments, including isothermal, square-waved, linear cooling, and exponential cooling temperature profiles. In general, the errors of computer simulation were within 0.5 $\log_{10}$ CFU/g. Such accuracy is sufficient for general food safety evaluation. Since this method developed in this study is capable of simulating and estimating bacterial growth under complicated temperature conditions, it can be used by the food industry to design proper cooling conditions and to estimate the extent of growth of *C. perfringens* in cooked meat products when there is a temperature deviation. Various regulatory agencies, food retailers, and consumers also can use the method developed in this study to evaluate the

microbial safety of food products based on the time–temperature history. Although the methodology and computational algorithm were developed and validated using *C. perfringens* as a test organism, they can be used to estimate other foodborne pathogens if their growth kinetics are available.

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